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IGF-I and GH post-receptor signaling mechanisms for pancreatic β -cell replication

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ABSTRACT

Certain nutrients, pharmacological agents and growth factors can stimulate pancreatic β -cell proliferation; however, mitogenic signal transduction pathways in β -cells have not been particularly well characterized. As a model system we have focussed on characterizing the signal transduction pathways immediately downstream of the IGF-I and GH receptors in β -cells. The original idea was to gain an idea of important elements in mitogenic signaling pathways which might then be exploited to generate a marked increase in β -cell proliferation. Such an approach could eventually reveal a means to increase the number of human pancreatic endocrine cells *in vitro*, in order to obtain an

abundant source of β -cells for routine transplantation therapy of type-I diabetes. However, in the course of our studies, we have also unveiled an unexpected insight into the pathogenesis of obesity-linked type-II diabetes. It has been observed that free fatty acids inhibit glucose- and glucose-dependent IGF-I/GH-induced β -cell proliferation. We hypothesize that a gradual accumulation of intracellular fat in β -cells during obesity can eventually lead to an inhibition of β -cell mass expansion and hence failure to compensate for peripheral insulin resistance, so that type-II diabetes ensues.

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INTRODUCTION

Pancreatic islet β -cell growth can be mediated by two separate mechanisms (Bonner-Weir 1992, Swenne 1992). Either new islets can form from budding of the pancreatic ductal epithelium (neogenesis) or from replication of existing islet β -cells (Bonner-Weir 1992). Neogenesis of islets primarily occurs during fetal and perinatal stages of development (Swenne 1992), but has also been observed in the regenerating adult pancreas (Smith *et al.* 1991). Replication of pancreatic β -cells has been observed in the late fetal stages, but it is thought to be the principal means of maintaining (or even increasing) β -cell mass in the adult (Bonner-Weir 1992, Swenne 1992). The replication rate of existing β -cells appears to be determined by the number of cells able to enter

into the cell division cycle (G1, S, G2 and M phases) from the quiescent G0 phase (Swenne 1992). However, in a population of normal adult pancreatic islet cells that are well differentiated, the number of β -cells actually undergoing cell division is small, measured to be between 0.5 and 2% (Swenne & Andersson 1984). Notwithstanding, it is possible to coerce adult β -cells into a proliferative state. Nutrients and several peptide growth factors have been shown to increase β -cell growth (Swenne 1992, Sjöholm 1997); however, the signal transduction pathways behind what leads a β -cell into replication have not been too well defined until recently. In this article, early signaling events in mitogenic signal transduction pathways of the pancreatic β -cell will be presented, using insulin-like growth factor-I (IGF-I) and growth hormone (GH) as 'model' growth factors.

FIGURE 1. IGF-I and GH signal transduction pathways. A schematic representation of key early elements in IGF-I and GH signal transduction pathways is shown. These pathways are described in detail in the text but also refer to Benito *et al.* (1996), Yenush & White (1997) and Carter-Su & Smit (1998) for excellent reviews. A solid arrow indicates a single step in a pathway and a broken arrow indicates that there are several more steps in the pathway that leads to a mitogenic event. pY indicates a phosphotyrosine, fyn, fyn-kinase (a Src-family tyrosine kinase); nck, a small SH2/SH3 domain containing adaptor protein; syp (or SHPTP2), a SH2-domain containing tyrosine phosphatase; 14-3-3, 14-3-3 proteins; T-Ag, SV40 large T-antigen; PKC ζ , protein kinase-C zeta isoform, and all other abbreviations are given in the text.

Atypical of most mammalian cells, but typical of the pancreatic β -cell through its continuous monitoring of nutrient status of the organism (Prentki 1996, Prentki *et al.* 1997), growth factor-induced β -cell proliferation is glucose dependent. Indeed, glucose itself is a β -cell mitogen and can independently activate selected mitogenic signal transduction pathways in the β -cell. Recent investigations into mitogenic signal transduction in β -cells have increased optimism about the prospect of obtaining large quantities of β -cells *in vitro* from adult human islets as an abundant source for transplantation therapy of type-I diabetes. Moreover, these studies have also gained some novel insights into the loss of control of β -cell mass expansion that is important to the pathogenesis of type-II diabetes.

IGF-I AND GH SIGNAL TRANSDUCTION PATHWAYS

IGF-I stimulates mammalian cell proliferation via the IGF-I receptor and subsequent activation of a protein tyrosine phosphorylation signal transduction cascade, generally similar to that of insulin action (Benito *et al.* 1996, Kadowaki *et al.* 1996). Briefly, and as outlined in Fig. 1, IGF-I binding to the IGF-I receptor activates the receptor's intrinsic tyrosine kinase activity, resulting in autophosphorylation and tyrosine phosphorylation of members of the insulin-receptor-substrate (IRS) family (Benito *et al.* 1996, Kadowaki *et al.* 1996). Migration of IRS to the plasma membrane is mediated via the plextrin-homology (PH) domain, and docking with the phosphotyrosines on the

IGF-I receptor β -subunit tyrosine kinase via the IRS phosphotyrosine-binding (PTB) domain (Yenush & White 1997). There are several members of the IRS family all with conserved N-terminal PH and PTB domains, but vary in variety and number of tyrosine phosphorylation Src-homology domain-2 (SH2-domain) docking sites in the C-terminal tails (see White 1998 for details). Tyrosine-phosphorylated IRS is then able to recruit the 85 kDa regulatory subunit of phosphatidylinositol-3'-kinase (PI3'-K) via its SH2 domain, leading to activation of the enzyme (Benito *et al.* 1996, Kadowaki *et al.* 1996, Yenush & White 1997) to convert phosphatidylinositol (PI(P)) into phosphatidylinositide (3,4,5) trisphosphate (PIP3). PIP3 then acts as a 'secondary messenger' to activate 3-phosphoinositide-dependent protein kinase-1 (PDK1) (Cohen 1999) and/or certain isoforms of protein kinase-C (PKC) (Liu 1996, Belham *et al.* 1999). PDK1 then activates protein kinase-B (PKB, also known as Akt) and certain atypical PKC isoforms by serine phosphorylation (Belham *et al.* 1999, Cohen 1999). Activated PKB then inactivates glycogen synthase kinase-3 (GSK3) via serine/threonine phosphorylation and, in contrast, activates another protein kinase, the mammalian target of rapamycin (mTOR), leading to downstream phosphorylation activation of the 70 kDa-S6 kinase (p70^{S6K}) (Myers *et al.* 1994, Belham *et al.* 1999). PKB can also directly serine/threonine phosphorylate certain transcription factors which, in turn, contribute to leading a cell into mitogenesis (Kane *et al.* 1999, Rena *et al.* 1999).

IGF-I-mediated tyrosine phosphorylation of IRS also engages the adaptor molecule, growth factor receptor-bound protein-2 (Grb2) via its SH2 domain to a phosphotyrosine site on IRS (Benito *et al.* 1996, Kadowaki *et al.* 1996). This, in turn, leads to an increased binding of IRS-docked Grb2 to the murine son-of-sevenless-1 protein (mSOS), a guanine nucleotide exchange factor which converts inactive Ras-GDP into active Ras-GTP (Benito *et al.* 1996, Kadowaki *et al.* 1996). Activated GTP-bound Ras then recruits the Raf serine kinase which, in turn, phosphorylates mitogen-activated protein (MAP)-kinase-kinase (MEK), resulting in MEK-mediated phosphorylation activation of the MAP kinase (MAPK; erk-1 and -2 isoforms) (Kadowaki *et al.* 1996). Activated MAPK can then activate other protein kinases such as the p90 ribosome subunit kinase (p90^{RSK}), or migrate to the nucleus where it may phosphorylate certain transcription factors, leading to mitogenesis (Khoo & Cobb 1997). IGF can also activate the Ras/MAPK branch of the pathway independently of IRS, via IGF-I receptor kinase tyrosine phosphorylation of

the SH2-containing protein (Shc) which then directly binds Grb2 to mSOS, resulting in activation of Ras/MAPK (Benito *et al.* 1996, Kadowaki *et al.* 1996). Notwithstanding, whichever signal transduction pathway is mediated, activation of MAPK and PI3'-K are known to be a requirement for induction of mitogenesis in most mammalian cell types (Benito *et al.* 1996, Kadowaki *et al.* 1996).

GH instigates a mitogenic signal in mammalian cells predominately via activation of the Janus kinase-2 (JAK2)/signal transducer and activation of transcription-5 (STAT5) signal transduction pathway (Carter-Su & Smit 1998). GH binds to the GH receptor causing dimerization, and then recruitment, tyrosine phosphorylation and activation of the tyrosine kinase, JAK2. JAK2 then phosphorylates STAT5 within a transient GH receptor/JAK2/STAT5 signaling complex from which phosphorylated STAT5 is released for migration to the nucleus where it affects gene transcription contributing to mitogenesis (Carter-Su & Smit 1998, Sekine *et al.* 1998).

GLUCOSE-INDUCED MITOGENESIS IN β -CELLS

Glucose can independently increase mitogenesis in primary pancreatic β -cells (Svenne 1992), for which glucose metabolism is required (Bonner-Weir 1992, Sjöholm 1996). Unlike the majority of eukaryotic cells, glucose-induced β -cell mitogenesis occurs over the physiologically relevant concentration range (6–18 mM) (Hügl *et al.* 1998, Cousin *et al.* 1999). However, the intracellular signaling pathways responsible for glucose-mediated β -cell proliferation, beyond a requirement for glucose metabolism, are not particularly well understood. Increases in glucose metabolism in β -cells can lead to rises in intracellular cAMP and subsequent protein kinase-A (PKA) activation, PKC activity and cytosolic intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) (Prentki & Matchinsky 1987, Prentki 1996, Sjöholm 1997), all of which have been implicated as potential secondary signals for glucose-induced β -cell growth (Bonner-Weir 1992, Svenne 1992, Hügl *et al.* 1998). It has been shown that glucose can independently give rise to increased Shc, IRS-1 and -2 tyrosine phosphorylation, leading to increased recruitment of Grb2/mSOS to Shc/IRS and downstream activation of MAPK (Khoo & Cobb 1997, Hügl *et al.* 1998). However, glucose-induced MAPK activation does not appear to correlate with a dose-response curve of glucose-induced β -cell proliferation, nor does inhibition of MEK prevent

glucose-induced DNA synthesis (Hügl *et al.* 1998, Cousin *et al.* 1999). As such, it is not clear that MAPK activation is required for glucose-induced β -cell mitogenesis. Glucose can also independently induce recruitment of PI3'-K to IRS-1 and -2, resulting in stimulation of PI3'-K activity and downstream activation of p70^{S6K} (Hügl *et al.* 1998, Cousin *et al.* 1999). In this instance, p70^{S6K} activation did correlate with glucose-induced β -cell proliferation in a dose-dependent manner which was completely blocked by inhibition of PI3'-K (Hügl *et al.* 1998, Cousin *et al.* 1999). As such, it is considered that the PI3'-K branch of IRS-mediated mitogenic signal transduction is essential for glucose-induced β -cell mitogenesis. In contrast to IRS-mediated signaling in β -cells, glucose could not independently activate JAK2/STAT5 signal transduction as utilized by GH (Cousin *et al.* 1999).

A question arises as to how increased glucose metabolism in β -cells gives rise to activation of IRS-mediated signal transduction pathways. Pyruvate and mitochondrial fuels (e.g. the combination of glutamate/leucine) can also promote β -cell proliferation (S P Cousin, S R Hügl & C J Rhodes, unpublished observations), so that secondary signals are likely to emerge from β -cell anaplerosis. These unidentified metabolic coupling signals will undoubtedly lead to activation of certain protein kinases, for phosphorylation activation of elements in IRS signaling, which include PKA, certain PKC isoforms and certain Ca²⁺/calmodulin-dependent kinases (although not Ca²⁺/calmodulin-dependent kinase-II (CaMK-II)) (Svenne 1992, Sjöholm 1996, Khoo & Cobb 1997, Hügl *et al.* 1998, Cousin *et al.* 1999). Moreover, since the tyrosine phosphorylation state of Shc, IRS-1 and -2 is increased by glucose, then glucose is also capable of influencing activation of a protein tyrosine kinase and/or inhibiting a phosphoprotein tyrosine phosphatase activity in β -cells. It should also be considered that glucose-induced activation of some signaling elements that require serine/threonine phosphorylation may bypass an upstream requirement for Shc/IRS-1/-2. For example, prevention of a glucose-induced rise in β -cell cytosolic [Ca²⁺]_i prevents glucose-induced MAPK activation (Khoo & Cobb 1997). This suggested that MAPK can be phosphorylated by Ca²⁺-dependent serine/threonine protein kinase. Future studies should elucidate which protein kinases are appropriate for glucose-induced activation of mitogenic signal transduction in β -cells. This will be of particular importance, since in pancreatic β -cells glucose provides a platform permissive for growth factor-induced β -cell proliferation (Svenne 1992, Hügl *et al.* 1998, Cousin *et al.* 1999).

GLUCOSE-DEPENDENT IGF-I-INDUCED MITOGENESIS IN β -CELLS

IGF-I is a significant stimulus for inducing pancreatic β -cell growth (Svenne 1992). Furthermore, IGF-I has been implicated as playing a prominent role in increasing the population of islet β -cells in the developing and regenerating pancreas (Smith *et al.* 1991, Bonner-Weir 1992). However, IGF-I appears only to be effective at inducing β -cell proliferation in the physiologically relevant glucose concentration range between 6 and 18 mM (Hügl *et al.* 1998). Thus, unlike the mitogenic effect of IGF-I on other eukaryotic cells (Benito *et al.* 1996), in pancreatic β -cells an IGF-I-induced mitogenic response is glucose dependent (Hügl *et al.* 1998, Cousin *et al.* 1999). IGF-I potentiates the mitogenic effect of glucose on β -cell proliferation. As such, there is a further increase in Shc, IRS-1 and -2 tyrosine phosphorylation, resulting in increased PI3'K association with IRS and Grb2/mSOS association with Shc and IRS by IGF-I at >6mM glucose. This results in increased downstream activation of MAPK (Erk-1 and -2 isoforms) and p70^{S6K} above that of glucose alone (Hügl *et al.* 1998, Cousin *et al.* 1999). As for glucose-induced β -cell proliferation, the PI3'K branch of IRS-mediated signal transduction is a necessity for glucose-dependent IGF-I-induced β -cell proliferation (Hügl *et al.* 1998). However, unlike in the absence of IGF-I, glucose dose-response activation of MAPK is also required for glucose-dependent IGF-I-induced β -cell DNA synthesis (Hügl *et al.* 1998). IGF-I has no effect on activating the JAK2/STAT5 signaling pathway (Cousin *et al.* 1999), nor activation of other MAPK family members, such as p38, and Jun kinases (JNK-1 and -2) (S P Cousin, S R Hügl & C J Rhodes, unpublished observations).

GLUCOSE-DEPENDENT GH-INDUCED MITOGENESIS IN β -CELLS

GH is a potent inducer of β -cell proliferation (Hellerström *et al.* 1991). It has been previously suggested that GH mediates increases in β -cell proliferation via local IGF-I production (Svenne *et al.* 1987). However, this is now considered unlikely, as GH predominantly induces β -cell proliferation via a JAK2/STAT5 signal transduction pathway (Argetsinger & Carter-Su 1996, Sekine *et al.* 1998, Cousin *et al.* 1999). Interestingly, as with IGF-I, GH-induced β -cell proliferation is also glucose dependent (Cousin *et al.* 1999). Glucose has no independent effect on JAK2/STAT5 activation, and

IRS-2 mRNA Expression Levels in Pancreatic β -cell Lines

FIGURE 2. IRS-2 is specifically over-expressed in pancreatic β -cell lines. Northern blot analysis of IRS-2 was carried out on 10 μ g total RNA extracted from isolated rat islets; New England Deaconess Hospital (NEDH) rat insulinoma tissue; insulin-producing cell lines: RINm5f, Rin 1042, β TC3, β TC6-F7, MIN-6, INS-1, RITz; glucagon-producing cell line aTC1; pituitary cell lines AtT20 and GH3; and the neuronal cell line PC12 as described by Schuppin *et al.* (1998). Difference in loading is compared with ethidium bromide staining of 28S RNA on the same gel. Compared with isolated islets and non-insulin-producing cell lines, IRS-2 is highly expressed in the proliferating β -cell lines.

it is as yet unclear how glucose provides a means to permit GH action, although it has been suggested that Ca^{2+} is a possible mediator (Sekine *et al.* 1998, Cousin *et al.* 1999). PI3'K activity, likely provided by glucose-induced activation, also appears to be required for GH to promote a β -cell proliferative response (Cousin *et al.* 1999). However, the GH signaling pathway does not directly cross talk to IGF-I signal transduction pathways in β -cells (Cousin *et al.* 1999), as it does in other eukaryotic cells (Argetsinger & Carter-Su 1996). As such, the glucose-dependent GH and IGF-I β -cell response is synergistic, giving a marked increase in β -cell proliferation (Cousin *et al.* 1999). If it is desired to increase β -cells *in vitro*, one should consider using at least both IGF-I and GH in combination. It will be important in future studies to investigate IGF-I/GH-induced proliferation in primary human pancreatic islet β -cells or a human β -cell line.

TYPE-I DIABETES: PROSPECTS AND POTENTIAL FOR GAINING ENOUGH HUMAN ISLET CELLS FOR β -CELL REPLACEMENT THERAPY

Type-I diabetes is a disease state where endogenous pancreatic β -cells have been lost by autoimmune destruction (Slover & Eisenbarth 1997). The pros and cons of pancreatic β -cell replacement/transplantation therapy have been described else-

where (Docherty 1991, Robertson 1992, Newgard 1994, Weir & Bonner-Weir 1997, Efrat 1998). Notwithstanding, human islet replacement can work quite well as illustrated by islet autograft transplantations (Robertson 1992). However, there are essentially two main stumbling blocks: (1) immune rejection of transplanted pancreatic β -cells in an unfriendly autoimmune environment of type-I diabetes, and (2) gaining enough human pancreatic islet β -cells for β -cell replacement therapy to become commonplace. Recent advances using novel immunosuppressive agents have increased optimism in overcoming rejection of islet grafts (Kenyon *et al.* 1999, Thomas *et al.* 1999). More relevant to this article though, and in the light of new insight into regulation of β -cell growth, it is worth commenting on whether human islet β -cells can be significantly increased *in vitro*, prior to transplantation. Several growth factors have been applied to isolated islets *in vitro*, but these do not have a dramatic effect on significantly increasing a β -cell population (Sjoholm 1996). Alternatively, it has been thought that if key elements in growth factor signal transduction pathways can be unveiled, then these might be exploited to better induce β -cell growth (Welsh *et al.* 1987). However, it was not until recently that such potential 'key elements' have been identified. A marked increased expression of IRS-2 has been found specifically in insulinoma cells (Schuppin *et al.* 1998), also found in the majority of insulin-producing cell lines (Fig. 2). This inversely

PATHOGENESIS OF TYPE-II DIABETES- A QUESTION OF BALANCE ?

FIGURE 3. An outline of the progression of type-II diabetes.

correlates with the observation in IRS-2 knockout mice where there is a marked decrease in β -cell mass and smaller islets (Withers *et al.* 1997). It has also been found that PI3'-K plays a pivotal role in glucose-dependent IGF-I and GH-induced β -cell proliferation (Hügl *et al.* 1998, Cousin *et al.* 1999). In early experiments, adenoviral-mediated over-expression of IRS-2 and constitutively active PI3'-K in glucose-sensitive INS-1 cells markedly increases glucose-dependent IGF-I induced β -cell proliferation (L Dickson, S R Hügl, B B Kahn, M G Myers & C J Rhodes, unpublished observations). Although this is encouraging, it has yet to be attempted in primary human islet cells. A degree of caution should be made, however, in that without careful control this approach may generate human insulinoma cell lines. Although these cells would be useful for research purposes and cell-based drug screening assays, they might not be suitable for insulin replacement transplantation therapy due to their tumorigenic properties and/or dedifferentiation state, resulting in an inability to correctly respond to glucose in a physiologically relevant manner (Efrat 1998). It is conceivable that such human β -cell lines could be 'bioengineered' into a

phenotype that better mimics primary β -cells (Newgard 1994), or even protected from cytokine-mediated destruction (Hohmeier *et al.* 1998), but progress has been slow in this area, since human β -cell lines have been unavailable. Recently, however, human pancreatic β -cell lines have been obtained from patients with a syndrome known as persistent hyperinsulinemic hypoglycemia of infancy (PHHI) (Macfarlane *et al.* 1999). Thus, there is reason for optimism that these PHHI β -cell lines might eventually be used in β -cell replacement therapy, especially since they can be bioengineered by transfecting in key β -cell-specific transcription factors which improve the differentiation state of the PHHI cells (Macfarlane *et al.* 1999). Nonetheless, one must still be cautious since proliferating human β -cell lines will remain somewhat dedifferentiated tumorigenic β -cell lines. Even if contained within a retrievable biocompatible membrane device (Weir & Bonner-Weir 1997), without a strategy to regain a somewhat differentiated state, proliferating human β -cell lines will carry a significant safety risk if transplanted. Actually, there is currently no clear-cut guidelines from government agencies, such as the Federal Drug

Administration in the United States, to advise on the use of such cell lines for transplantation therapy, which, if available, would markedly improve progress in this area. Nonetheless, alternative approaches to generate mature differentiated pancreatic β -cells by manipulation of key transcription factors (Docherty 1991, Yamaoka & Itakura 1999), or from sources such as pancreatic ductal cells (Corbett *et al.* 1997), embryonic stem cells (Kim & Melton 1998), and fetal islet clusters (Beattie *et al.* 1997) are being considered, but these ' β -cells' will also need to be produced in relatively large quantities. Thus, similar considerations to expand such β -cells *in vitro* with minimal dedifferentiation as described above will also apply to these ' β -cells' if they are eventually to be used for transplantation purposes. However, with ever-improving gene transfer technologies and a better understanding of β -cell mitogenic signal transduction pathways, it is possible that the goals of *in vitro* human β -cell expansion could soon be met (Efrat 1998).

TYPE-II DIABETES: PATHOGENESIS AT THE LEVEL OF PREVENTING β -CELL GROWTH

There is a growing consensus as to the pathogenesis of obesity-linked type-II diabetes in which regulation of pancreatic β -cell growth plays a central role. This is outlined in Fig. 3. Under normal circumstances, there is a balance between β -cell mass and requirements for insulin action in the periphery. In a prediabetic phase, peripheral insulin resistance is compensated for by an increased β -cell mass, perhaps caused by transient hyperglycemia and glucose-induced β -cell growth. However, as time goes on, with increasing hyperglycemia and hyperlipidemia the β -cell mass is no longer able to compensate for the insulin resistance and even decreases (Unger 1995). Therefore, loss of control for pancreatic β -cell growth and/or death is a key to the pathogenesis of type-II diabetes. It has been suggested that both hyperglycemia and hyperlipidemia can cause β -cell apoptosis, causing a reduction in β -cell mass (Pick *et al.* 1998, Shimabukuro *et al.* 1998). In addition, at glucose concentrations >18 mM, glucose and glucose-dependent IGF-I and GH-induced β -cell proliferation are reduced. This suggests that prolonged hyperglycemia could contribute to reducing β -cell mass by also inhibiting β -cell growth (Hügl *et al.* 1998, Cousin *et al.* 1999). We have recently found that fatty acids (FFA) inhibit glucose, and glucose-dependent IGF-I-induced β -cell proliferation (Fig. 4). The half-time for FFA-induced inhibition was between 6 and 8 h using 0.2 mM palmitate or oleate

FIGURE 4. FFAs inhibit glucose- and glucose-dependent IGF-I-induced β -cell proliferation. The rate of β -cell proliferation at 15 mM glucose $\pm$ 10 nM IGF-I was determined by accessing [3 H]thymidine incorporation into glucose-sensitive INS-1 cells which had been previously incubated for 24 h with various palmitate concentrations complexed to 1% BSA, as previously described (Hügl *et al.* 1998, Cousin *et al.* 1999). Results are expressed as a fold increase above the control in the absence of glucose (mean $\pm$ S.E., $n \geq 6$). Increasing palmitate concentrations inhibited both 15 mM glucose and 15 mM glucose + 10 nM IGF-I-induced β -cell proliferation. Similar effects were also observed with oleate and linoleic acid instead of palmitic acid.

complexed to 1% bovine serum albumin (BSA), and in a 24-h period half maximal FFA-induced inhibition was around 0.08 mM palmitate or oleate complexed to 1% BSA. It is likely that FFA-induced inhibition of β -cell proliferation, together with FFA-induced β -cell apoptosis (Shimabukuro *et al.* 1998), can reduce β -cell mass *in vivo*. Upon examination of glucose and IGF-I signal transduction pathways, it was found that glucose/IGF-I induced Grb2/mSOS and PI3'K recruitment to IRS-1 and -2, and Grb2/mSOS recruitment to Shc was unaffected by FFA. Likewise, neither glucose/IGF-I-induced MAPK nor PI3'K activation were affected by FFA. However, FFA rendered a 50% inhibition on glucose/IGF-I-induced activation of PKB and chronically activated PKC ζ . It remains to be shown directly whether inhibition of PKB and/or

chronic activation of PKC ζ prevent glucose/IGF-I-induced β -cell proliferation. However, it is interesting to note that activation of certain PKC isoforms and inhibition of PKB has been implicated in FFA-induced insulin resistance (Griffin *et al.* 1999, Schmitz-Peiffer *et al.* 1999). Considering that IGF-I and insulin share comparable signal transduction pathways (Kadowaki *et al.* 1996), similar FFA interference of insulin signal transduction in insulin target tissues and IGF-I signaling in pancreatic β -cells can contribute to both insulin resistance and the decline in β -cell mass in the pathogenesis of type-II diabetes (Withers *et al.* 1999).

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